# Genome-Wide Linkage Analysis to Identify Chromosomal Regions Affecting Phenotypic Traits in the Chicken. IV. Metabolic Traits[1]

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**ABSTRACT** The current study is a comprehensive genome analysis to detect QTL affecting metabolic traits in chickens. Two unique $F_2$ crosses generated from a commercial broiler male line and 2 genetically distinct inbred lines (Leghorn and Fayoumi) were used in the present study. The plasma glucagon, insulin, lactate, glucose, triiodothyronine, thyroxine, insulin-like growth factor I, and insulin-like growth factor II concentrations at 8 wk were measured in the 2 $F_2$ crosses. Birds were genotyped for 269 microsatellite markers across the entire genome. The program QTL Express was used for QTL detection. Significance levels were obtained using the permutation test. For the 10 traits, a total of 6 and 9 significant QTL were detected at a 1% chromosome-wise significance level, of which 1 and 6 were significant at the 5% genome-wise level for the broiler-Leghorn cross and broiler-Fayoumi cross, respectively. Most QTL for metabolic traits in the present study were detected in Gga 2, 6, 8, 9, 13, and Z for the broiler-Leghorn cross and Gga 1, 2, 4, 7, 8, 13, 17, and E47 for the broiler-Fayoumi cross. Phenotypic variation for each trait explained by all QTL across genome ranged from 2.73 to 14.08% in the broiler-Leghorn cross and from 6.93 to 21.15% in the broiler-Fayoumi cross. Several positional candidate genes within the QTL region for metabolic traits at the 1% chromosome-wise significance level are biologically associated with the regulation of metabolic pathways of insulin, triiodothyronine, and thyroxine.

**Key words**: genome scan, quantitative trait loci, metabolic trait, broiler, inbred line

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## INTRODUCTION

The study of circulating protein hormones and metabolites has long been of interest to both the biomedical and animal agricultural communities. Since the discovery of insulin (**INS**) in 1921 and its subsequent production in swine and cattle to treat diabetes, the study of hormones and their regulation of metabolism has been the focus of vast research efforts. Initially, these efforts focused on determining the underlying hormonal basis of disease and eventually defined the functional pathways for how many hormones affect metabolic homeostasis. It is this information that sparked the interest in hormonal regulation of metabolism in domestic species whose growth, feed efficiency, and health are of significant economic importance to animal agriculture.

The inactivation of specific hormone genes leads to dramatic shifts in hormone levels and their related metabolites (O'Shea and Williams, 2002; Robson et al., 2002), but little is known about the natural genetic variation present and its contribution to the variation in normal circulating hormone concentrations. Previous genome-wide studies in rodents (Rosen et al., 2000; Suto and Sekikawa, 2002; Almind et al., 2003; Anunciado et al., 2003; Harper et al., 2003), swine (Desautes et al., 2002), and humans (Santos et al., 2004; Sonnenberg et al., 2004) have identified QTL for specific hormone concentrations specifically related to disease states. Park et al. (2006) detected several QTL affecting metabolic traits including Glc, INS, and INS-like growth factor- (**IGF**) I in an intercross generated from chicken lines divergently selected for growth. The current study investigated the natural variation present in the genomes of a novel inbred-outbred cross resource population to determine if QTL exist for circulating INS, glucagon (**GLG**), glucose (**Glc**), lactate (**LCT**), IGF-I, IGF-II, and thyroid hormones triiodothyronine ($\mathbf{T_3}$) and thyroxine ($\mathbf{T_4}$).

Insulin and GLG are produced by the pancreas in response to circulating Glc concentrations and act hormonally to regulate Glc metabolism and storage in the form of GLG (Hazelwood, 1984). These hormones act antago-



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**Table 1.** The partial correlations between metabolic traits in the $F_2$ population ($P < 0.05$)

| Trait[1] | IGR | INS | $T_4$ | $T_3$ | $T_3$:$T_4$ | LCT | Glc | IGF-I | IGF-II |
|---|---|---|---|---|---|---|---|---|---|
| GLG | –0.683 | –0.339 | 0.058† | –0.450 | –0.365 | 0.213 | 0.078 | 0.210 | 0.481 |
| IGR | — | 0.759 | 0.004† | 0.321 | 0.245 | –0.178 | –0.086 | –0.122 | 0.312 |
| INS | — | — | 0.066† | 0.256 | 0.184 | –0.155 | –0.003† | –0.038† | –0.261 |
| $T_4$ | — | — | — | –0.215 | –0.626 | 0.118 | 0.082 | 0.062† | –0.037† |
| $T_3$ | — | — | — | — | 0.836 | –0.348 | –0.028† | –0.087 | –0.501 |
| $T_3$:$T_4$ | — | — | — | — | — | –0.324 | –0.066† | –0.098 | –0.362 |
| LCT | — | — | — | — | — | — | 0.579 | 0.299 | 0.322 |
| Glc | — | — | — | — | — | — | — | 0.427 | 0.102 |
| IGF-I | — | — | — | — | — | — | — | — | 0.402 |

[1]GLG = glucagon; INS = insulin; LCT = lactate; IGR = INS:GLG; $T_3$ = triiodothyronine; $T_4$ = thyroxine; Glc = glucose; IGF = insulin-like growth factor.

†$P > 0.05$.

nisticly; INS activates the storage of Glc in the form of glycogen, and GLG activates the breakdown of glycogen to Glc. The 2 hormones therefore act in concert to maintain Glc homeostasis.

In periods of activity, circulating plasma LCT levels are elevated due to the metabolism of Glc and glycogen in the muscle and incomplete oxidation to pyruvate. At steady state, circulating LCT is indicative of protein turnover or lack in uptake in peripheral tissues (Ashwell and McMurtry, 2003).

Insulin-like growth factor-I is produced primarily in the liver in response to pituitary-derived growth hormone. Insulin-like growth factor-I has been shown to regulate growth, reproduction, energy balance, cell proliferation, and cell death (McMurtry et al., 1997). Animal agriculture has focused much research on IGF-I as a potential enhancer of lean muscle growth (Duclos et al., 1999). Conversely, IGF-II is primarily expressed in the developing embryo but is activated in mature animals during periods of stress. The $T_3$ and $T_4$ hormones are produced by the thyroid gland and are key regulators of basal metabolism via effects on the mitochondrial respiratory pathway. Effects on the mitochondrial uncoupling protein directly influence heat production and thus metabolic activity (Dridi et al., 2004).

Previous analysis of the parental lines and the resulting $F_2$ phenotypic data of the populations investigated in the present study indicated significant genetic variation is present in the chicken-affecting hormone and metabolite concentrations and that the effect is polygenic (Ashwell et al., 2002). The major objective of the present study was to detect and localize QTL affecting metabolic traits in the 2 unique $F_2$ populations.

## MATERIALS AND METHODS

### Resource Populations

The Iowa Growth and Composition Resource Population was established by crossing sires from a broiler breeder male line with dams from genetically distinct, highly inbred (>99%) chicken lines, the Leghorn G-B2 and Fayoumi M15.2 (Zhou and Lamont, 1999; Deeb and Lamont, 2002). The $F_1$ birds were intercrossed, within dam line, to produce 2 related $F_2$ populations. Birds (n = 417 in broiler-Leghorn cross; n = 325 in broiler-Fayoumi cross) of the 2 $F_2$ populations were analyzed, with each population representing progeny from 1 broiler grandsire and 1 $F_1$ sire of each cross.

### Phenotypic Measurements

Blood samples were collected in EDTA-treated tubes from 8-wk-old birds before euthanizing, and plasma was transferred into tubes containing 1,000 IU of trasylol as a preservative. Plasma concentrations of INS (McMurtry et al., 1983), $T_3$, and $T_4$ (McMurtry et al., 1988) were measured using a double antibody RIA. Plasma GLG was measured using an RIA kit purchased from Linco Research Inc. (St. Charles, MO) as described previously (Ashwell et al., 2002). A double antibody RIA was used to measure plasma concentrations of IGF-I, with an intraassay CV of 2.6% (McMurtry et al., 1994), and chicken IGF-II, with an intraassay CV of 3.6% (McMurtry et al., 1998). Plasma Glc and LCT were measured by specific electrode analysis (YSI 2700 SELECT Biochemistry Analyzer, YSI Inc., Yellow Springs, OH).

**Table 2.** The significant 5 and 1% chromosome-wise level, as determined by permutation test, for metabolic traits by chromosome in the broiler-Leghorn cross and the broiler-Fayoumi cross

| | Broiler-Leghorn cross | | Broiler-Fayoumi cross | |
|---|---|---|---|---|
| Gga | 5% | 1% | 5% | 1% |
| 1 | 7.65 | 10.38 | 7.48 | 10.00 |
| 2 | 5.99 | 8.06 | 7.24 | 10.00 |
| 3 | 6.66 | 8.60 | 6.23 | 8.19 |
| 4 | — | — | 5.54 | 7.66 |
| 5 | 5.97 | 8.46 | — | — |
| 6 | 5.00 | 6.95 | 4.97 | 6.86 |
| 7 | — | — | 5.23 | 7.86 |
| 8 | 4.62 | 6.56 | — | — |
| 9 | 5.40 | 7.65 | — | — |
| 11 | 4.96 | 7.16 | — | — |
| 13 | 4.86 | 6.89 | 5.44 | 7.59 |
| 15 | 5.31 | 7.69 | — | — |
| 17 | 4.19 | 6.04 | 5.21 | 7.56 |
| 18 | — | — | 4.64 | 7.05 |
| E47 | — | — | 4.49 | 7.67 |
| Z | 4.81 | 6.67 | 4.80 | 6.65 |

**Table 3.** Evidence for QTL significant at the 5% chromosome-wise level for metabolic traits by chromosome in the broiler-Leghorn cross[1]

| Gga | Trait[2] | *F*-value | Location | Additive effect | SE | Dominance effect | SE | Variance (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | $T_4$ | 7.98 | 751 | 0.91 | 0.37 | 1.69 | 0.42 | 4.09 |
| 1 | IGF-I | 8.59 | 102 | 4.18 | 1.21 | 2.29 | 2.93 | 4.39 |
| 2 | INS | 12.61** | 353 | –0.45 | 0.09 | –0.09 | 0.15 | 6.30 |
| 3 | $T_4$ | 7.23 | 327 | –0.41 | 0.12 | 0.43 | 0.19 | 3.72 |
| 3 | $T_3$ | 7.12 | 436 | 0.52 | 0.14 | 0.24 | 0.18 | 3.67 |
| 3 | $T_3$:$T_4$ | 8.5 | 432 | 7.58 | 1.84 | 3.93 | 2.61 | 4.35 |
| 3 | IGF-I | 8.48 | 379 | –3.14 | 0.76 | 0.95 | 1.12 | 4.34 |
| 5 | IGF-II | 5.7 | 0 | –17.89 | 4.89 | –15.32 | 5.26 | 3.46 |
| 6 | IGR | 5.01 | 52 | –10.08 | 3.19 | –10.33 | 3.39 | 2.61 |
| 6 | INS | 9.28* | 54 | –1.81 | 0.42 | –1.79 | 0.43 | 4.73 |
| 6 | $T_4$ | 6.7 | 41 | 0.61 | 0.17 | 0.20 | 0.22 | 3.46 |
| 6 | IGF-II | 5.16 | 0 | 25.58 | 7.98 | –19.81 | 8.09 | 2.68 |
| 8 | INS | 5.71 | 68 | 0.27 | 0.09 | –0.30 | 0.14 | 2.96 |
| 8 | $T_3$ | 7.17* | 34 | 0.56 | 0.24 | 0.27 | 0.25 | 2.74 |
| 8 | $T_3$:$T_4$ | 5.97 | 33 | 8.01 | 3.72 | 4.41 | 3.82 | 3.09 |
| 8 | LCT | 7.66* | 37 | –184.06 | 50.08 | –212.51 | 54.45 | 3.94 |
| 8 | Glc | 5.25 | 30 | 403.43 | 125.17 | 381.27 | 127.37 | 2.73 |
| 8 | IGF-I | 5.77 | 33 | –36.10 | 11.65 | –29.59 | 11.96 | 2.99 |
| 9 | IGR | 5.84 | 162 | 6.63 | 1.99 | –8.24 | 2.51 | 3.03 |
| 9 | INS | 5.88* | 164 | 0.83 | 0.25 | –0.98 | 0.30 | 3.05 |
| 11 | $T_3$:$T_4$ | 5.77 | 18 | 6.43 | 2.06 | 4.41 | 2.20 | 2.74 |
| 13 | GLG | 7.81* | 35 | 189.57 | 48.97 | –203.60 | 56.08 | 4.01 |
| 13 | LCT | 6.28 | 18 | –79.90 | 22.70 | 49.67 | 27.61 | 3.25 |
| 15 | $T_3$ | 5.81 | 19 | 0.35 | 0.17 | 0.03 | 0.18 | 3.01 |
| 15 | $T_3$:$T_4$ | 6.6 | 38 | 8.24 | 2.30 | –2.74 | 2.53 | 3.41 |
| 17 | INS | 4.78 | 102 | 0.16 | 0.09 | 1.61 | 0.63 | 2.49 |
| 17 | IGF-I | 4.53 | 102 | –1.81 | 0.89 | –13.38 | 5.98 | 2.37 |
| Z | IGR | 6.67* | 8 | –2.81 | 0.90 | 1.95 | 0.90 | 3.44 |

[1]Estimated significance levels (*F*-value), location, gene effects, and percentage of $F_2$ variance explained by each QTL.

[2]GLG = glucagon; INS = insulin; LCT = lactate; IGR = INS:GLG; $T_3$ = triiodothyronine; $T_4$ = thyroxine; Glc = glucose; IGF = insulin-like growth factor.

*Significant at 1% chromosome-wise level; **significant at 5% genome-wise level ($F > 10.26$).

### Marker Selection, Genotyping, Linkage Analysis, and QTL Mapping

All birds were genotyped for 269 markers as described by Zhou et al. (2006). The marker linkage analysis and QTL mapping used were as described in Zhou et al. (2006). Significance levels at the 5 and 1% chromosome-wise and the 5 and 1% genome-wise levels were determined by permutation as described by Zhou et al. (2006).

### Partial Correlation Analysis

The phenotypic correlations between metabolic traits were obtained using the JMP program (Sall and Lehman, 1996). Each partial correlation was simultaneously adjusted for all other variables than the 2 being compared.

## RESULTS

### Phenotypic Correlations Between Metabolic Traits

The phenotypic correlations between metabolic traits in the combined 2 $F_2$ populations are presented in Table 1. There were general high correlations between each 2 traits, except that there were relative low correlations between $T_4$, Glc, and IGF-I with most of the other traits. The $T_4$ had relative high correlations with $T_3$ and $T_3$:$T_4$, as expected, and relative low correlations with all others except LCT. There were relative high correlations between Glc and LCT, IGF-I, and IGF-II.

### Significance Thresholds

Individual chromosome significance levels at the 5 and 1% level, as determined by the permutation test, differed slightly by trait within chromosome (Table 2). Average 5% chromosome-wise thresholds ranged from 4.19 to 7.65 in the broiler-Leghorn cross and from 4.49 to 7.48 in the broiler-Fayoumi cross. Average 1% chromosome-wise thresholds ranged from 6.04 to 10.38 in the broiler-Leghorn cross and from 6.65 to 10.00 in the broiler-Fayoumi cross. Average 5 and 1% genome-wise thresholds were 10.26 and 13.66, respectively, in the broiler-Leghorn cross and were 10.71 and 14.45, respectively, in the broiler-Fayoumi cross.

### General QTL Mapping Results

Estimates for QTL significant at the 5% chromosome-wise level are presented in Tables 3 and 4. The QTL graphs, representing plots of the *F*-statistic across chromosomes, are presented in Figures 1 and 2. Although some graphs suggest evidence for multiple QTL in adja-

**Table 4.** Evidence for QTL significant at the 5% chromosome-wise level for metabolic traits by chromosome in the broiler-Fayoumi cross[1]

| Gga | Trait[2] | *F*-value | Location | Additive effect | SE | Dominance effect | SE | Variance (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | $T_3$ | 14.64*** | 72 | –0.25 | 0.05 | –0.19 | 0.09 | 8.66 |
| 1 | $T_3$:$T_4$ | 16.99*** | 70 | –5.75 | 1.03 | –4.04 | 1.75 | 9.91 |
| 1 | IGF-I | 12.94** | 543 | –12.42 | 2.82 | –15.25 | 3.02 | 7.73 |
| 2 | Glc | 10.87** | 441 | –225.12 | 48.40 | –81.32 | 66.85 | 6.57 |
| 3 | $T_3$:$T_4$ | 7.12 | 204 | 6.24 | 1.75 | –6.09 | 3.47 | 4.41 |
| 4 | IGR | 6.87 | 91 | 4.81 | 1.31 | –2.05 | 1.48 | 4.25 |
| 4 | $T_4$ | 11.15** | 257 | 0.60 | 0.33 | 1.53 | 0.64 | 6.73 |
| 4 | Glc | 6.25 | 295 | 140.17 | 63.68 | –393.76 | 122.23 | 3.89 |
| 4 | IGF-I | 7.15 | 106 | 2.56 | 1.17 | –5.59 | 1.62 | 4.42 |
| 4 | IGF-II | 5.77 | 247 | –2.70 | 3.08 | –13.95 | 6.20 | 4.05 |
| 5 | IGF-II | 5.7 | 0 | –17.89 | 4.89 | –15.32 | 5.26 | 3.46 |
| 6 | IGR | 6.57 | 68 | –0.33 | 1.04 | –5.79 | 1.60 | 4.08 |
| 6 | INS | 6.47 | 67 | –0.24 | 0.13 | –0.67 | 0.21 | 4.01 |
| 7 | GLG | 6.4 | 91 | –276.13 | 78.91 | –249.11 | 80.19 | 3.97 |
| 7 | Glc | 12.5** | 130 | –727.18 | 178.93 | 256.45 | 182.81 | 7.48 |
| 13 | GLG | 9.86* | 68 | 125.48 | 40.07 | –124.80 | 40.64 | 6.00 |
| 17 | GLG | 6.11 | 57 | 38.09 | 37.22 | 156.07 | 44.97 | 3.81 |
| 17 | IGR | 8.22* | 63 | –2.71 | 1.57 | –7.0 | 1.95 | 5.05 |
| 17 | INS | 7.05 | 69 | –0.18 | 0.15 | –0.61 | 0.18 | 4.36 |
| 18 | LCT | 5.4 | 4 | –25.86 | 10.50 | 33.89 | 16.35 | 3.38 |
| E47 | LCT | 8.45* | 17 | 194.39 | 67.84 | –219.23 | 66.16 | 5.12 |
| Z | LCT | 5.28 | 39 | 38.35 | 12.31 | 14.52 | 12.30 | 3.30 |
| Z | Glc | 5.47 | 5 | –110.59 | 52.16 | 124.14 | 52.74 | 3.41 |

[1]Estimated significance levels (*F*-value), location, gene effects, and percentage of $F_2$ variance explained by each QTL.

[2]GLG = glucagon; INS = insulin; LCT = lactate; IGR = INS:GLG; $T_3$ = triiodothyronine; $T_4$ = thyroxine; Glc = glucose; IGF = insulin-like growth factor.

*Significant at 1% chromosome-wise level; **significant at 5% genome-wise level ($F > 10.71$); ***significant at 1% genome-wise level ($F > 14.45$).

cent intervals for the same trait, only results for the most significant position are presented in Tables 3 and 4, because only single QTL models were tested.

In total, 28 and 23 QTL were detected at the 5% chromosome-wise level for the 10 traits examined in the broiler-Leghorn cross and the broiler-Fayoumi cross, respectively, not counting potential multiple QTL in adjacent intervals. Ten QTL would be expected to be significant at suggestive threshold by chance alone, given the 10 traits examined. Therefore, about 3 and 2 times as many QTL were detected at this level than were expected by chance in the broiler-Leghorn cross and the broiler-Fayoumi cross, respectively. Of the 28 suggestive QTL in the broiler-Leghorn cross, 1 QTL was significant at the 5% genome-wise level (Table 5). Of the 23 suggestive QTL in the broiler-Fayoumi cross, 6 QTL were significant at the 5% genome-wise level (Table 5). Over the 10 traits examined, 0.5 QTL would be expected to be significant at this level by chance alone. Thus, 10-fold more QTL were identified at this level than were expected. The QTL affecting metabolic-related traits were only identified on Gga 1, 2, 3, 5, 6, 8, 9, 11, 13, 15, 17, and Z in the broiler-Leghorn cross and on Gga 1, 2, 3, 4, 6, 7, 13, 17, 18, E47, and Z in the broiler-Fayoumi cross (Table 2).

The phenotypic trait variances explained by individual QTL ranged from 2.37 to 6.30 in the broiler-Leghorn cross and from 3.30 to 9.91 in the broiler-Fayoumi cross (Tables 3 and 4).

***GLG.*** For the broiler-Leghorn cross, 1 QTL for GLG was detected on Gga 13 (Table 3). The additive effect suggested that broiler alleles were superior to the Leghorn alleles. The QTL showed overdominance, and heterozygotes concerning breed origin of microsatellite had lower GLG than either of the homozygotes (Table 3). For the broiler-Fayoumi cross, 3 QTL for GLG were identified on Gga 7, 13, and 17 (Table 4). Broiler alleles tended to be associated with higher GLG than the Fayoumi alleles, except for the QTL on Gga 7 (Table 4). One of the 3 QTL showed a high degree of overdominance, and heterozygotes had higher Glc than either of the homozygotes (Gga 17; Table 4). The total trait variances explained by QTL for GLG were 4.01 and 13.78% in the broiler-Leghorn and broiler-Fayoumi crosses, respectively (Table 5).

***INS.*** For the broiler-Leghorn cross, 5 QTL effects on INS were detected on Gga 2, 6, 8, 9, and 17 (Table 3). Broiler alleles were superior to Leghorn alleles for 3 of 5 QTL (Table 3). Heterozygotes had the greatest INS:GLG (**IGR**) for 1 of 5 QTL. For the broiler-Fayoumi cross, 2 QTL for INS were identified on Gga 6 and 17, with Fayoumi alleles resulting in greater INS (Table 4). Heterozygotes showed lower INS than either of the homozygotes (Table 4). The total trait variances explained by QTL were 19.53 and 8.37% in the broiler-Leghorn and broiler-Fayoumi crosses, respectively (Table 5).

***IGR.*** For the broiler-Leghorn cross, 3 QTL for IGR were identified on Gga 6, 9, and Z (Table 3). Leghorn alleles tended to be associated with higher IGR than the broiler alleles, except for the QTL on Gga 9 (Table 3). One of the 3 QTL showed overdominance (Gga 6), and 1 showed complete dominance (Gga 9). Heterozygotes had lower

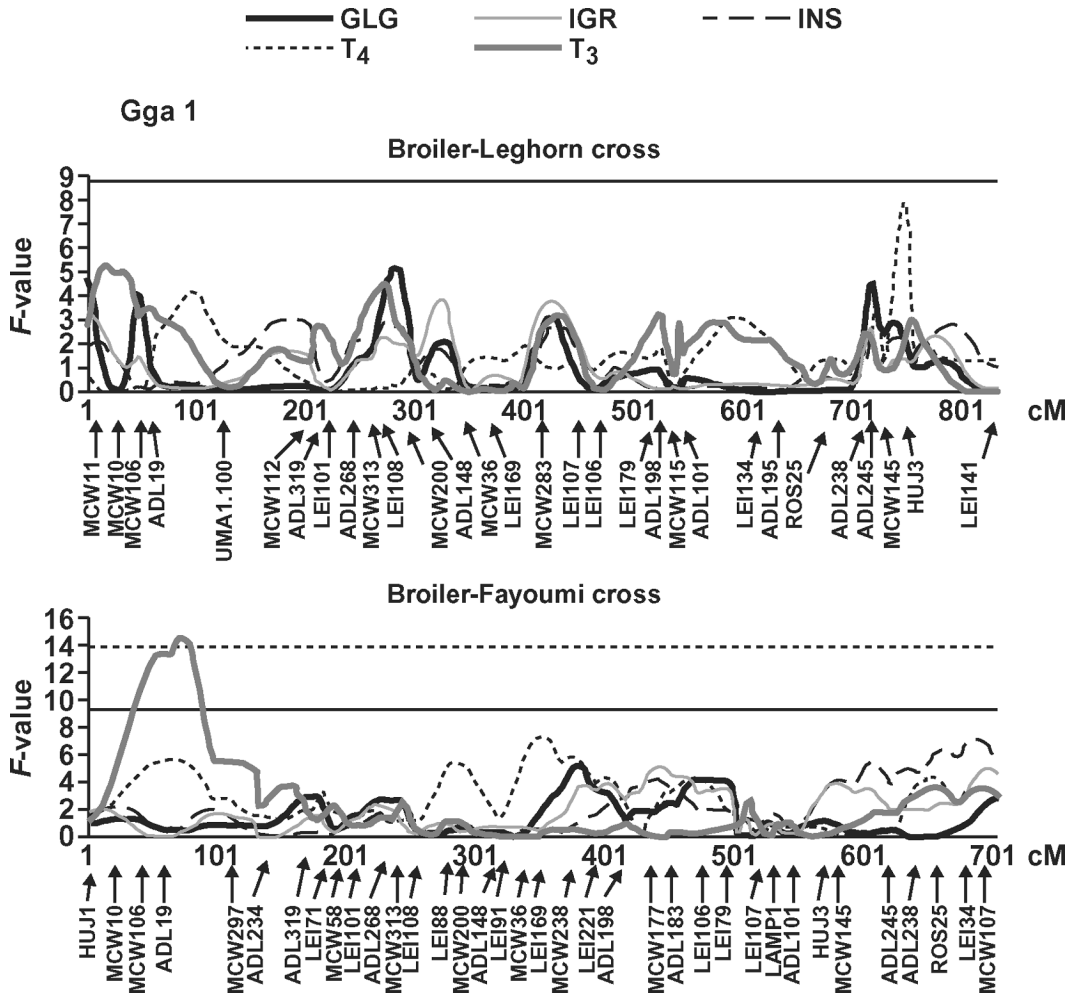


**Figure 1.** The *F*-value curves for evidence of QTL for glucagon (GLG), insulin (INS), INS:GLG (IGR), thyroxine ($T_4$), and triiodothyronine ($T_3$) traits. The x-axis indicates the relative position on the linkage group. The y-axis represents the *F*-value. Arrows on the x-axis indicate the positions where a marker was present. Two lines are provided for 1% chromosome-wise (—) and 1% genome-wise (- - - -) significance.

IGR than either of the homozygotes in the QTL with overdominance effect (Table 3). For the broiler-Fayoumi cross, 3 QTL for IGR were identified on Gga 4, 6, and 17 (Table 4). The additive effect suggested that Fayoumi alleles were superior to the broiler alleles, except for the QTL on Gga 4. Two of 3 QTL had overdominance effect, and heterozygotes had lower IGR than either of the homozygotes (Table 4). The total trait variances explained by QTL were 9.08 and 13.38% in the broiler-Leghorn and broiler-Fayoumi crosses, respectively (Table 5).

***$T_4$, $T_3$, and $T_3$:$T_4$.*** In total, 10 QTL affecting $T_4$, $T_3$, and $T_3$:$T_4$ were found on Gga 1, 3, 6, 8, 11, and 15 (Table 3). The additive effect suggested that broiler alleles were superior to the Leghorn alleles, except for the QTL for $T_4$ on Gga 3 (Table 3). Heterozygotes showed the greatest $T_4$ at QTL on Gga 1. The results revealed 4 suggestive QTL on Gga 1, 3, and 4 in the broiler-Fayoumi cross (Table 4). Broiler alleles were superior to the Fayoumi alleles for 2 out of the 4 QTL. One of the 4 QTL showed overdominance effect, and the heterozygote showed greater $T_4$ than either of the homozygotes (Gga 4). The total trait variances explained by QTL for $T_4$, $T_3$, and $T_3$:$T_4$ were 11.27, 9.42, and 13.59% in the broiler-Leghorn and 6.73, 8.66, and 14.32% in the broiler-Fayoumi crosses, respectively (Table 5).

***LCT.*** Two QTL were detected for LCT on Gga 8 and 13 in the broiler-Leghorn cross (Table 3). Leghorn alleles showed associations with greater LCT than the broiler alleles. One of 2 QTL showed overdominance (Table 3). Three QTL were identified on Gga 18, E47, and Z in the broiler-Fayoumi crosses (Table 5). Broiler alleles showed higher LCT than the Fayoumi alleles, except for the QTL on Gga 18. The results indicated overdominance in 2 out of 3 QTL. The total trait variances explained by QTL were 7.19 and 11.80% in the broiler-Leghorn and broiler-Fayoumi crosses, respectively (Table 5).

***Glc.*** One QTL was found on Gga 8 for Glc in the broiler-Leghorn cross, whereas 4 QTL were identified on Gga 2, 4, 7, and Z (Tables 3 and 4). The additive effect indicated that broiler alleles were superior to the Leghorn alleles or Fayoumi alleles for 2 of the 5 QTL. Two of the 5 QTL showed overdominance, and heterozygotes had greater Glc than either of the homozygotes (Gga Z) and lower Glc (Gga 4; Table 4). These effects accounted for nearly

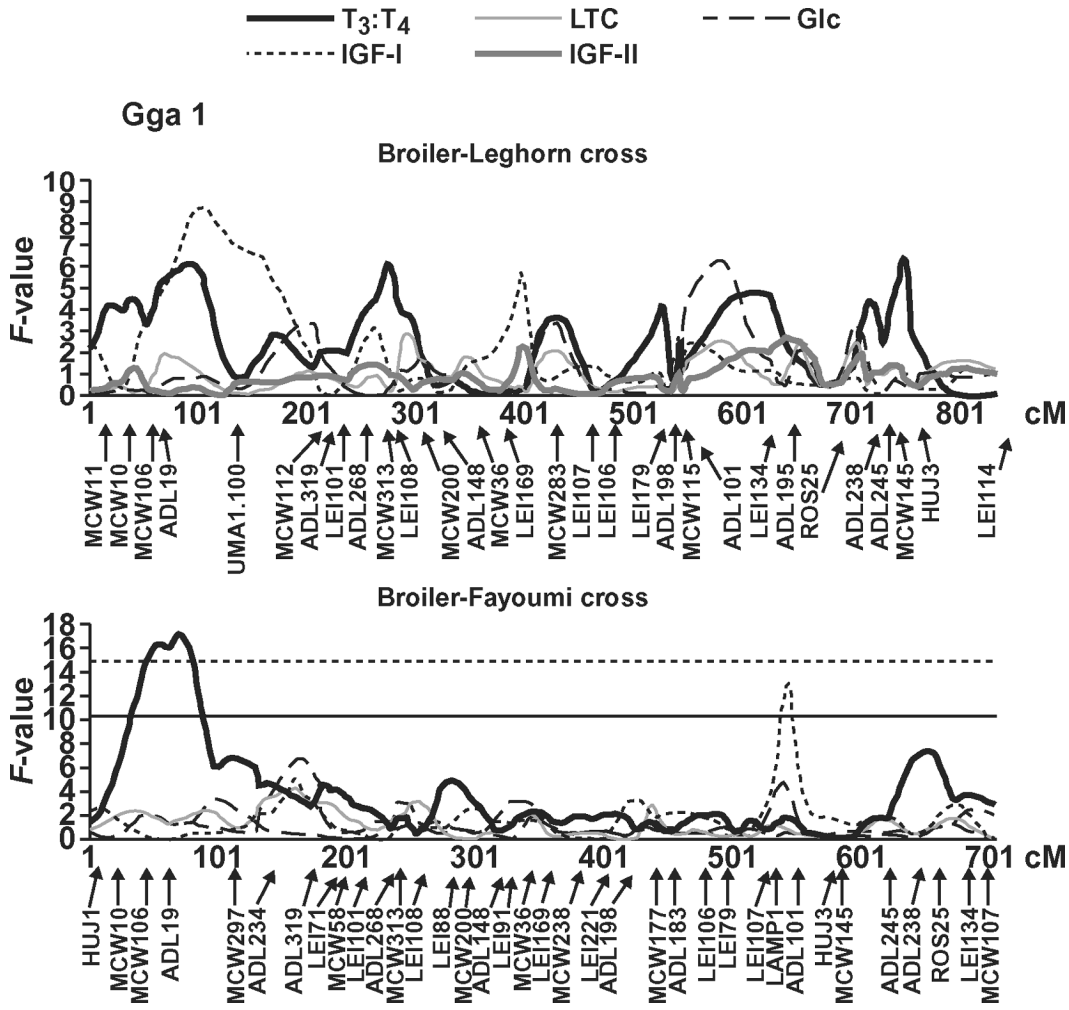


**Figure 2.** The *F*-value curves for evidence of QTL for triiodothyronine:thyroxine ($T_3$:$T_4$), lactate (LCT), glucose (Glc), insulin-like growth factor-(IGF) I and IGF-II traits. The x-axis indicates the relative position on the linkage group. The y-axis represents the *F*-value. Arrows on the x-axis indicate the positions where a marker was present. Two lines are provided for 1% chromosome-wise (—) and 1% genome-wise (- - - -) significance.

2.73 and 21.35% of the variation in the broiler-Leghorn and broiler-Fayoumi crosses, respectively (Table 5).

***IGF-I and IGF-II.*** Six QTL were detected for IGF-I and IGF-II on Gga 1, 3, 6, 8, and 17 in the broiler-Leghorn cross (Table 3). Leghorn alleles showed associations with greater IGF-I or IGF-II than the broiler alleles for 4 of the 6 QTL. One of 6 QTL showed strong overdominance for IGF-I (Table 3). Heterozygotes had lower IGF-I than either of the homozygotes. Four QTL were identified on Gga 1, 4, and 5 in the broiler-Fayoumi cross (Table 4). Broiler

**Table 5.** Number of QTL significant at the 5 and 1% chromosome-wise level (CHR) and genome-wise (GEN) level, respectively, by trait in $F_2$ broiler-Leghorn and broiler-Fayoumi crosses

| | Broiler-Leghorn cross | | | | | Broiler-Fayoumi cross | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trait[1] | 5% CHR | 1% CHR | 5% GEN | 1% GEN | Variance (%) | 5% GEN | 1% GEN | 5% GEN | 1% GEN | Variance (%) |
| GLG | — | 1 | — | — | 4.01 | 2 | 1 | — | — | 13.78 |
| IGR | 2 | 1 | — | — | 9.08 | 2 | 1 | — | — | 13.38 |
| INS | 1 | 1 | 1 | — | 14.08 | 2 | — | — | — | 8.37 |
| $T_4$ | 3 | — | — | — | 11.27 | | — | 1 | — | 6.73 |
| $T_3$ | 2 | 1 | — | — | 9.42 | | — | — | 1 | 8.66 |
| $T_3$:$T_4$ | 3 | — | — | — | 10.84 | 1 | — | — | 1 | 14.32 |
| LCT | 1 | 1 | — | — | 7.19 | 2 | 1 | — | — | 11.80 |
| Glc | 1 | — | — | — | 2.73 | 2 | — | 2 | — | 21.35 |
| IGF-I | 3 | — | — | — | 11.10 | 1 | — | 1 | — | 12.15 |
| IGF-II | 3 | — | — | — | 9.13 | 2 | — | — | — | 7.51 |

[1]GLG = glucagon; INS = insulin; LCT = lactate; IGR = INS:GLG; $T_3$ = triiodothyronine; $T_4$ = thyroxine; Glc = glucose; IGF = insulin-like growth factor.

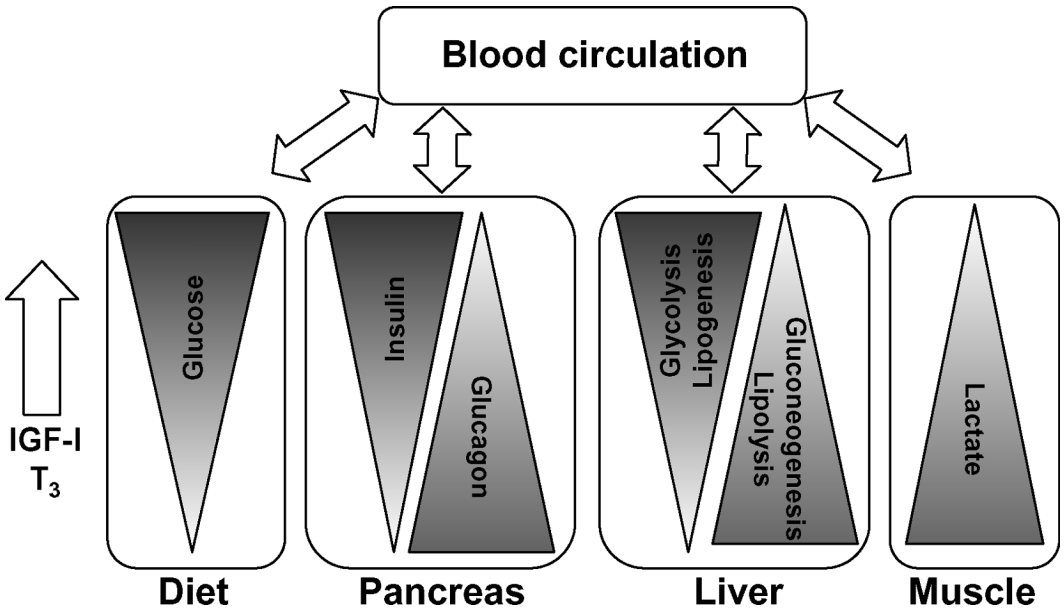


**Figure 3.** Schematic of the relationships between circulating metabolic factors and metabolism in endocrine and energy-producing and utilizing tissues. As glucose (Glc) levels rise (from bottom to top), insulin levels rise and glycolysis and lipogenesis predominate. When blood Glc levels fall, glucagon increases and metabolism shifts to gluconeogenesis and lipolysis. Triiodothyronine ($T_3$) and insulin-like growth factor-1 (IGF-I) both stimulate the shift of metabolism toward glycolysis and induce insulin production.

alleles showed lower IGF-I or IGF-II than the Fayoumi alleles, except for the QTL for IGF-I on Gga 4. The current results indicated overdominance in all 4 QTL. Heterozygotes had the lowest IGF-I or IGF-II for all 4 QTL. The total trait variances explained by QTL for IGF-I and IGF-II were 11.10 and 9.13% in the broiler-Leghorn and 12.15 and 7.51% in the broiler-Fayoumi crosses, respectively (Table 5).

## DISCUSSION

In the present study, 9 metabolic factor traits were analyzed for QTL using a whole genome scan. A total of 51 QTL were detected in the 2 related $F_2$ crosses at the 5% chromosome-wise significance level. Of these QTL, 14 were significant at the 1% chromosome-wise level. Those QTL (Table 6) with the highest significance levels will be discussed further.

The regulation of metabolism in higher animals is a complex series of checks and balances that must respond to environmental stimuli including diet (nutrition) and temperature. Metabolic regulation follows patterns of response to nutrient availability that results from either feeding or some other stimulus such as physical activity. In general, metabolic response to feeding follows the schematic outlined in Figure 3. As dietary energy increases, primarily supplied as carbohydrate following a meal, blood Glc concentrations increase. Five QTL (Gga 2, 4, 7, 8, and Z) affecting plasma Glc concentrations were identified in the present study, but none of them appeared in the QTL positions (Gga 20 and 27) identified in a previous study (Park et al., 2006).

The increase in blood Glc following a meal stimulates the release of INS by the B-islet cells of the pancreas. Insulin stimulates the uptake and storage of this energy by the liver in the form of glycogen and lipid by also stimulating lipogenic enzymes (Hazelwood, 1984). Upon the reduction of blood Glc concentrations either by fasting or increased energy demand, to maintain homeostasis, the pancreas produces GLG, which stimulates the mobilization of energy from both glycogen and lipid stores to supply the peripheral tissues. This negative energy balance often follows physical activity, whereby the muscle tissues utilize LCT as an energy source for gluconeogenesis (Juel, 1997). These processes rely on feedback mechanisms to maintain body homeostasis, whereby the IGR is maintained (Maruyama et al., 1984).

A family of significant QTL for INS, GLG, and IGR were also detected (Table 6). The first of these QTL was located on Gga 2 in the marker interval of MCW264 to GCT2. Although 2 QTL affecting INS (Gga 1 and 2) have been reported in chickens (Park et al., 2006), the QTL for INS in the present study has not been described previously. There are several positional candidate genes in this region, including high-Glc regulated protein-8 and corticotrophin-releasing hormone (**CRH**). The high-Glc regulated protein-8 is a gene that responds to hyperglycemia and is of interest clinically in diabetic nephropathy and may represent a therapeutic target for the complex disease (Lappin et al., 2002). It is logical that this gene may elicit some effects on INS concentrations, but there is no current evidence for their direct interaction.

The location of CRH in an interval containing a QTL for circulating INS is also logical, because energy homeostasis is regulated by neuropeptides including CRH. The response to CRH is anorexigenic, limiting feeding behavior (Hillebrand et al., 2002). The CRH itself is regulated by feeding behavior and is therefore cycling in a similar

**Table 6.** Positional candidate genes for metabolic QTL identified in both the Leghorn and Fayoumi crosses

| Gga | Location | Line[1] | Trait[2] | Positional candidate gene(s)[3] | Described previously |
|---|---|---|---|---|---|
| 1 | MCW10-MCW106 | FAY | $T_3$, $T_3$:$T_4$ | Insulin growth factor I precursor, phosphodiesterase ID | Vasilatos-Younken et al., 1999; de Lange et al., 2001; Schmid et al., 2003 |
| 2 | MCW264-GCT2 | LEG | INS | High-Glc regulated protein 8, corticotrophin-releasing protein | — |
| 2 | ADL181-BCL2 | FAY | Glc | Insulin receptor, succinate dehydrogenase | — |
| 4 | LEI73-ADL203 | FAY | $T_4$ | SH3 domain-binding protein | — |
| 6 | ADL377 | LEG | INS | Pancreatic lipase-related protein I precursor | — |
| 7 | ADL109 | FAY | Glc | Glucagon precursor | — |
| 8 | MCW147 | LEG | $T_3$, LCT | Type I iodothyronine deiodinase | Cogburn et al., 1997 |
| 9 | MCW135-MCW329 | LEG | INS | Adiponectin | — |
| 13 | ADL310-MCW213 | LEG, FAY | GLG | NADH dehydrogenase, Toll-like receptor 2 precursor | — |
| 17 | ADL293-ADL202 | FAY | IGR | Prostaglandin E synthase | — |
| 20 (E47) | LEI80 | FAY | LCT | Transforming growth factor $\beta$-induced transcription factor 2 | — |
| Z | MCW258-ADL273 | LEG | IGR | MAP-ERK kinase kinase 1, MEK kinase 1 | — |

[1]FAY = broiler-Fayoumi cross; LEG = broiler-Leghorn cross.

[2]GLG = glucagon; INS = insulin; LCT = lactate; IGR = INS:GLG; $T_3$ = triiodothyronine; $T_4$ = thyroxine; Glc = glucose; IGF = insulin-like growth factor.

[3]MAP = mitogen-activated protein; ERK = extracellular signal-regulated kinases; MEK = MAP-ERK kinase.

manner to INS after a meal is consumed. It has been shown that CRH inhibits INS secretion and increases GLG secretion in rodents (Karlsson and Ahren, 1988).

Additional QTL for INS concentrations were detected on Gga 6 and 9 in marker regions for ADL377 and MCW135 to MCW329, respectively. These QTL have not been previously reported in these regions for chickens, but there are positional candidate genes present that may contribute to INS concentrations. The pancreatic lipase-related protein I precursor gene is located on Gga 6, and the adiponectin gene is located on Gga 9, both near the QTL for INS. The pancreatic lipase-related protein I is an important mediator of INS action in peripheral tissues (Kintscher and Law, 2005). It has also been associated with gestational diabetes, where INS resistance occurs (Thyfault et al., 2005). Insulin resistance can be described as the general case in broiler-type chickens (Seki et al., 2003). The QTL on Gga 9 has a positive effect from the broiler allele, further supporting adiponectin, an adipocytokine, or adipose tissue signaling cytokine, as a positional candidate gene, possibly for affecting INS resistance as observed in broilers.

In addition to blood Glc, the production of INS is also stimulated by the hormones IGF-I and the thyroid hormone $T_3$ (Decuypere et al., 2005). Thyroid hormones also regulate other metabolic processes in response to cold temperature or other stress (Wentworth and Ringer, 1986). The production of $T_4$ and $T_3$ in the thyroid is diagramed in Figure 4. The main hormone that is produced by the thyroid is $T_4$, although some $T_3$ is made. The bioactive thyroid hormone is $T_3$, which is produced from deiodination of $T_4$ by deiodinase in the periphery (Freeman and McMabb, 1991). Therefore, $T_3$:$T_4$ is relevant to the metabolic status of the animal. Increased $T_3$ upregulates basal metabolism ($O_2$ consumption) with a corresponding decrease in growth rate, due to allocation of metabolic resources (energy; Wentworth and Ringer, 1986). The $T_3$ also affects carbohydrate metabolism by stimulating INS production and energy storage as glycogen (Decuypere et al., 2005).

Gga 1 contains a QTL for $T_3$ and $T_3$:$T_4$ in the interval of markers MCW10 to MCW106. Other groups have previously observed QTL in this region for early growth traits (Carlborg et al., 2003) as well as for the behavior trait tonic immobility (Schütz et al., 2004) in Leghorn-Jungle Fowl crosses. Both of these traits may be related to the thyroid status of the bird due to $T_3$ and $T_4$ effects on basal metabolism (de Lange et al., 2001) and their general effects on growth and development (O'Shea and Williams, 2002). Upon querying the chicken whole genome draft sequence in this region for genes that may possibly be related to $T_3$ or $T_4$ concentrations, several positional candidates were found. The IGF-I gene is in this region. It is known that IGF-I can be regulated by $T_3$ concentrations in birds and other vertebrates (Vasilatos-Younken et al., 1999; Schmid et al., 2003). An additional QTL for $T_3$ concentrations was found on Gga 8 in the

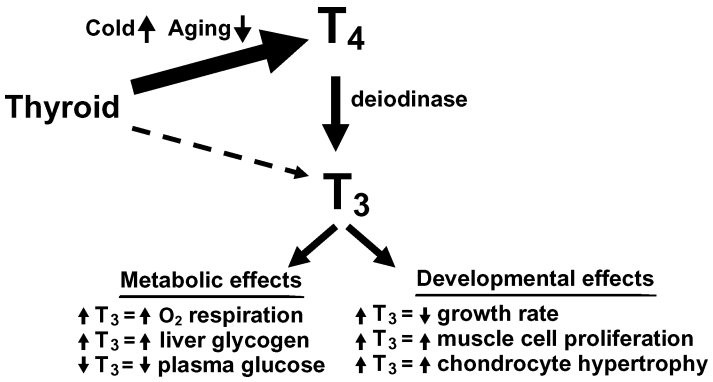


**Figure 4.** Schematic for the factors that influence the production of thyroid hormones and their effects on metabolism and development. The primary hormone produced by the thyroid is thyroxine ($T_4$), whereas some triiodothyronine ($T_3$) is also produced, as indicated by the arrow sizes.

broiler-Leghorn cross. This QTL was located where a previous group identified QTL for egg production traits (Tuiskula-Haavisto et al., 2002). This chromosomal region also contains the gene for the type I iodothyronine deiodinase enzyme. This enzyme is responsible for conversion of $T_3$ to $T_4$ and is involved in regulating $T_3$:$T_4$. The expression of this gene linked to growth hormone production possibly might explain the positive contribution of the broiler alleles at this locus (Cogburn et al., 1997). The deiodinase is an ideal positional candidate gene whose circulating substrate levels appear to be associated with $T_3$:$T_4$ as detected by QTL.

A QTL for $T_4$ levels was found on Gga 4 in the interval from LEI73 to ADL203. This region has been described by many groups as having effects on growth and egg production traits (Tuiskula-Haavisto et al., 2002; de Koning et al., 2003). There are a few positional candidate genes in the region including the SH3 domain-binding protein gene, which interacts with SH3 domains in cell-signaling protein kinases.

The QTL identified in this resource population represent a comprehensive study of association of metabolic factors and hormones with genetic loci in chickens. Associations between these circulating factors and performance traits have been previously shown, specifically for IGF-I and the thyroid hormones $T_3$ and $T_4$ (Tona et al., 2004). By identifying specific allele variants associated with circulating levels of these hormones, the cost of selecting for specific trends in the concentrations of these hormones (higher or lower) may be accomplished by genotyping at a significantly lower cost than performing the hormone assays. These QTL loci may also be useful in other species as determinants associated with metabolic status or hormone levels.

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